

FURTHER IMPROVEMENT UPON MAINTENANCE OF ADULT SQUID (*DORYTEUTHIS BLEEKERI*) IN A SMALL CIRCULAR AND CLOSED-SYSTEM AQUARIUM TANK

GEN MATSUMOTO AND JUNICHI SHIMADA

Electrotechnical Laboratory, Optoelectronics Section, Tsukuba Science City, Ibaraki 305, Japan

The squid *Loligo*, *Doryteuthis* and *Sepioteuthis* have large nerve fibers that make them particularly amenable to experimentation (Arnold *et al.*, 1974). However, difficulties in squid experiments result from regional and seasonal limitations on the availability of natural squid stocks. These limitations can be partially eliminated if the squid are maintained in an aquarium in the laboratory (Matsumoto, 1976).

We tried maintenance of adult squid at our laboratory located at Tanashi, a 3–5 hr drive from the fishing site near Jogashima Island, Kanagawa Prefecture (Matsumoto, 1976). We adopted a closed system with a circular tank where sea water was circulated peripherally along the tank wall to mitigate head-on collisions of the squid with the wall. We adopted this approach mainly because of the conclusions of Summers and McMahon (1974), and Summers *et al.*, (1974), that skin damage resulting from collisions with tank walls was a major factor limiting the survival of the squid in a small (*e.g.*, 1.68 m²) tank. Similar closed seawater systems with tank capacities of 1000 and 10,000 l were used by Hanlon *et al.* (1978) to maintain *Loligo plei* and *Loligo pealei*, with mean survival times of 13–25 days. O'Dor *et al.* (1977) obtained 32–82 day survival of specimens of *Illex illecebrosus* by mitigating collisions using a 15-m-diameter circular tank in an open system. We maintained squid (*Doryteuthis bleekeri*) in our closed system tank for as long as 2 weeks. We concluded that filtering ability was essential to squid survival in our closed system, and that skin damage was minor among causes of mortality in a 2-week maintenance period (Matsumoto, 1976). The question became: Can survival be improved if filtering ability is increased?

MATERIALS AND METHODS

The source of squid (*Doryteuthis bleekeri*), method of transporting them, and the aquarium system used in these experiments was similar to the one described in Matsumoto (1976): It consisted of an inner and an outer circular tank, three filtering stages, a charcoal filter, a dirt trap, a circulation pump, a temperature controller, and an air bubbler (Fig. 1). The outlet was directed so that filtered sea water flowed peripherally along the circular walls. Net patterns were drawn with black vinyl paint on the inner and the outer tank walls (Fig. 2). The peripherally circulating flow and these net patterns were particularly useful in reducing squid collisions with the tank walls (Matsumoto, 1976). Inner and outer tank walls were light brown polyethylene. Their diameters were 1.5 and 0.5 m, respectively, and their depth 1.2 m. Filtering stage 3, the charcoal filter, dirt trap, circulation pump, and temperature controller were the same as in Matsumoto (1976). Filtering stage 1 was composed of layers of commercially available zeolite and sand. The average diameter of zeolite grains was about 3 mm, and the thickness of the layer 15 cm. The

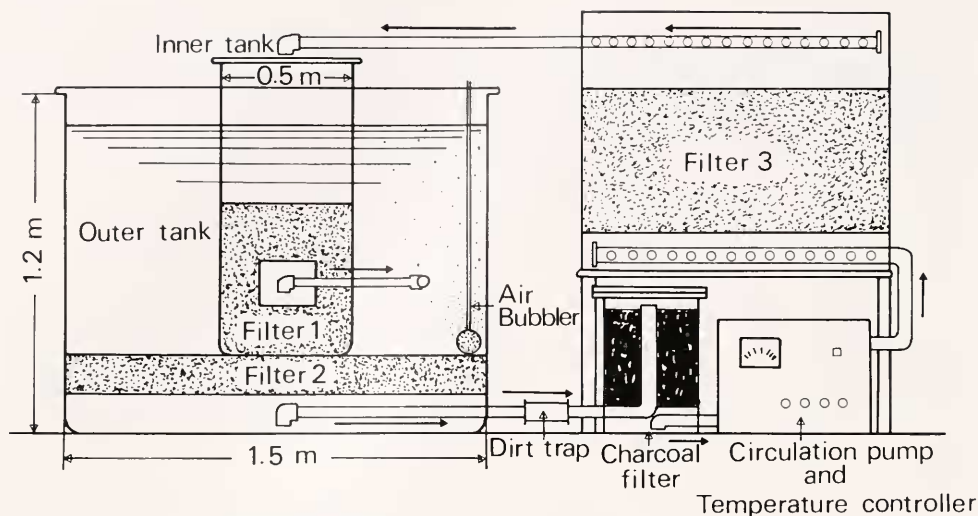


FIGURE 1. Configuration of the aquarium system. The arrows show the sequence of recirculating sea water through the system.

average diameter of the sand grains was 2 mm and the layer's thickness in most runs was 20 cm. The zeolite in the filter weighed 20 kg. In our previous system (Matsumoto, 1976) filter 1 contained a 5-cm layer of zeolite. Filtering stage 2 was the same as described in our previous report, except that 10 kg of crushed oyster shell was used to cover the filter surface. No direct sunlight entered the room when our laboratory was located at Tanashi, Tokyo. After our move to Tsukuba, Ibaraki prefecture, a 6–12 hr drive from the fishing site near Jogashima Island, the aquarium system was roofed to protect it from the rain but direct sunlight reached the system, resulting in the growth of greenish algae on the tank walls. At Tanashi and Tsukuba, a 40 W incandescent light about 1 m above the aquarium was kept lit day and night to help the squid see the tank walls.

Maintenance conditions were the same as those in Matsumoto (1976). Sea water flowed at the rate of 20 l/min through the system, and sea water temperature was kept between 15° and 18°C by a temperature controller. When squid were transferred to the aquarium from a transportation tank, aquarium temperature was adjusted to that in the tank. After transfer the temperature of sea water in the aquarium was lowered at less than 0.5°C/hr to between 15° and 18°C.

RESULTS

Maintenance of unfed squid

In November and December, 1978, we tried three runs of 2-week maintenance of squid (*Doryteuthis bleekeri*) by putting 15, 18, and 20 squid, respectively, into the aquarium without feeding them. The three runs resulted in no natural deaths, but eight squid were killed by cannibalism. No cannibalism was observed during first 3 days after the squid were transported from the fishing site.

Survival was appreciably improved as compared with that in our previous experiment (see the dashed line in Fig. 3). The system and maintenance conditions had not been changed, except for the replacement of the sand by 20 kg zeolite in filter 1 and the addition of oyster shell. The shell alone did not appreciably

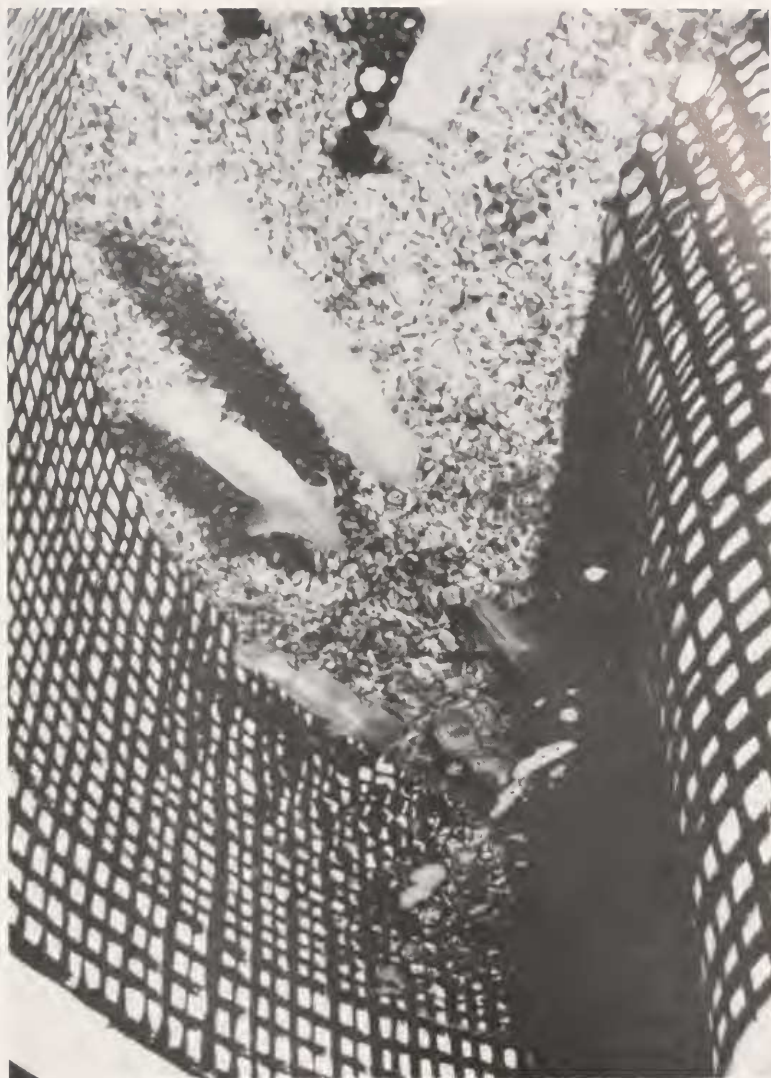


FIGURE 2. Tank walls' painted net patterns helped squid avoid collisions with the walls.

improve survival. When the thickness of zeolite layer in filter 1 was reduced to the original 5 cm, squid survival became similar to that of our previous experiment. Thus, improved survival was a function of zeolite. To further test the effect of zeolite upon survival, we tried three runs of 15, 12, and 5 squid, respectively, with filter 1 containing 80 kg zeolite. Average survival was 4.5 days (thin solid line in Fig. 3). Thus, 80 kg zeolite may be harmful to the squid. Probably, there exists an optimum amount of zeolite for survival. In our system, 20–40 kg of zeolite appeared optimum among amounts tested: 5, 20, 40, and 80 kg.

Maintenance of fed squid

Longer maintenance of squid (*Doryteuthis bleckeri*) by feeding them was tried February through April, 1979. Ten squid (one female, the rest male) were placed

in the aquarium at the end of February. Four to five red goldfish (*Carassius auratus*) 4–5 cm in length per squid were put into the tank for food every day after the third day from the beginning of the experiment. Feeding was conducted twice a day, at 9 a.m. and 5 p.m. At first the fish were not eaten, but after about a week, squid ate the fish within 30 sec after the fish were put into the aquarium.

Figure 3 shows survival of 10 squid over a 2-month span. The survival times were 43–60 days. The first squid (female) died after spawning. Five squid that survived for over a month died shortly after their skins were scraped at the mantle tips. Once this area of skin was scraped, the squid rubbed their mantle tips more frequently on the tank walls. Another experience indicated that even a slight injury at the tip was fatal: When we stapled a small mark sheet on the squid to discriminate old from new squids, those stapled at their mantle tips died sooner.

DISCUSSION

Our previous report (Matsumoto, 1976) suggested that the cause of death of squid in a closed-system aquarium, even with enough oxygen dissolved in the sea water, was primarily the squids' lack of oxygen-adsorbing ability. Our present experiment further suggests that an obstacle to oxygen adsorption is organic substances generated in the aquarium or a substance secondarily produced from these organic substances, which can be overcome by using zeolite in a filter. In maintaining squid (*Illex illecebrosus*) for more than 30 days in an open-system tank of 15 m in diameter, O'Dor *et al.* (1977) reported that sea water was pumped from the Northwest Arm of Halifax Harbor through intakes located at a depth of 15 m, 0.7 m off the bottom, and that the water quality was relatively high.

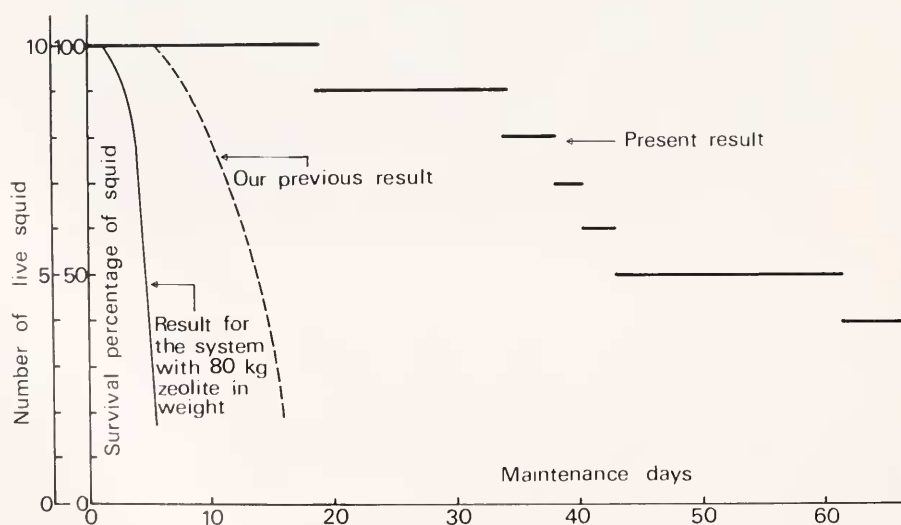


FIGURE 3. Survival of 10 squid over 2 months. The dashed line shows an old record of squid survival over 8 days (Matsumoto, 1976). The fine solid line shows results obtained for the system in Figure 1 when filter 1 contained 80 kg of zeolite. Calibration of squid survival record (discontinuous solid line) is in numbers of live squid, while calibrations for both fine solid and dashed lines (continuous lines) are in percentage of squid surviving.

With a closed or open system for maintenance of squid in the aquarium, high quality sea water seems to be crucial for squid survival.

In squid maintained for more than a month, skin damage was found to be fatal. In our maintenance system, the peripherally circulating flow of sea water, net patterns drawn on the tank walls, and continuous light enabled the squid to avoid hitting the walls. However, skin damage was caused by faint but repeated contacts with the tank walls, rather than by a harsh collision with the wall as suggested by Summers *et al.* (1974). Flores *et al.* reported that mortality after a 10-day survival test of squid (*Todarodes*) in an open system was high with the autumn-captured squid, possibly because of spread of a skin infection. O'Dor *et al.* (1977) concluded that deaths during the first week after specimens of *Illex illecebrosus* were put into the tank were associated with skin damage during capture. The reason why skin abrasion is fatal is not yet well understood. One explanation may be that skin abrasion provides an invasion site for bacteria, resulting in a disease leading to a quick death (Leibovitz *et al.*, 1977; Hulet *et al.*, 1979).

The condition of the squid when they were put into the tank obviously affected survival, as concluded by Summers *et al.* (1974) and O'Dor *et al.* (1977). In this respect, our squid-capturing method using Japanese squid jiggers (Matsumoto, 1976) is satisfactory. Initial condition also depends upon time between capture and arrival at a laboratory. In our case, shipboard transportation required 3–10 hr, and trucking from the seashore to our laboratories at Tanashi and Tsukuba took 3–5 and 6–12 hr, respectively. When truck transport took more than 8 hr, some squid were found to lie down on the bottom of the transportation tank. They usually recovered in the aquarium, but died within a week.

Two-week survival tests showed the importance of feeding squid. We tried live red goldfish and frozen sardines as food, and found the goldfish more suitable. Small frozen sardines, 9–12 cm long, were purchased from a fish seller. One sardine per squid a day was defrosted, tied at the tail by white thread and hung in the aquarium until captured by squid. The fatty sardines made the sea water oily, so that we had to make the sea water overflow by adding reserved sea water to the aquarium. Goldfish (*Carassius auratus*) usually live in fresh water, and do not survive in sea water longer than 10 min. However, squid were able to catch the live goldfish within 30 sec after the fish were put in the aquarium. The live goldfish were cheap (about 4 cents each), and squid can swallow them, so that sea water is kept clean.

Efforts to provide a steady supply of squid for neurosciences should now be directed to rearing from eggs in the aquarium (LaRoe, 1971; von Boltzky, 1971).

SUMMARY

Ten adult squid (*Doryteuthis bleekeri*) were put into a small (1.37 m² in area) circular tank in a closed system. Half of the squid survived 43–60 days, the other half longer. Filtering ability was essential to squid maintenance, and filtering ability was satisfactorily supported by zeolite in appropriate amounts. In long-term (over a month) maintenance, skin damage caused by faint but repeated contacts with the tank walls became a major cause of death. Feeding squid was found to be important for squid maintenance, and live goldfish (*Carassius auratus*) were found to be satisfactory as food for long-term maintenance of squid.

LITERATURE CITED

- ARNOLD, J. M., W. C. SUMMERS, D. L. GILBERT, R. S. MANALIS, N. W. DAW, AND R. J. LASEK, 1974. *A guide to the laboratory use of the squid Loligo pealei*. Marine Biological Laboratory, Woods Hole, Massachusetts. Pp. 9-17.
- FLORES, E. E. C., S. IGARASHI, AND T. MIKAMI, 1977. Studies on squid behavior in relation to fishing. II. On the survival of squid, *Torarodes pacificus Steenstrup*, in experimental aquarium. Bull. Fac. Fish. Hokkaido Univ., **28**: 137-142.
- HANLON, R. T., R. F. HIXON, AND W. H. HULET, 1978. Laboratory maintenance of wild-caught loliginid squids. *Fisheries and Marine Service Technical Report*, **833**: 20. 1-20.14.
- HULET, W. H., M. R. VILLOCH, R. F. HIXON, AND R. T. HANLON, 1979. Fin damage in captured and reared squids. Lab. Anim. Sci. **29**: 528-533.
- LEIBOVITZ, L., T. R. MEYERS, R. ELSTON, AND P. CHANEY, 1977. Necrotic exfoliative dermatitis of captive squid (*Loligo pealei*). *J. Invertebr. Pathol.*, **30**: 369-376.
- LAROE, E. T., 1971. The culture and maintenance of the loliginid squids *Sepioteuthis sepioidea* and *Doryteuthis plei*. *Mar. Biol.*, **9**: 9-25.
- MATSUMOTO, G. 1976. Transportation and maintenance of adult squid (*Doryteuthis bleekeri*) for physiological studies. *Biol. Bull.*, **150**: 279-285.
- O'DOR, R. K., R. D. DURWARD, AND N. BALCH, 1977. Maintenance and maturation of squid (*Illex illecebrosus*) in a 15 meter circular pool. *Biol. Bull.*, **153**: 322-335.
- SUMMERS, W. C., AND J. J. McMAHON, 1974. Studies on the maintenance of adult squid (*Loligo pealei*). I. Factorial survey. *Biol. Bull.*, **146**: 279-290.
- SUMMERS, W. C., J. J. McMAHON, AND G. N. P. A. RUPPERT, 1974. Studies on the maintenance of adult squid (*Loligo pealei*). II. Empirical extensions. *Biol. Bull.*, **146**: 291-301.
- VON BOLETZKY, S., M. V. VON BOLETZKY, D. FROSCH, AND V. GOTZI. 1971. Laboratory rearing of Sepiolinae (Mollusca: Cephalopoda). *Mar. Biol.*, **8**: 82-87.